CYCLIC PEPTIDE SURFACE FEATURE MIMICS OF ENDOTHELIN

This application is a Continuation-in-part of U.S. application Ser. No. 08/223,513, filed Apr. 5, 1994, now abandoned, and also a Continuation-in-part of U.S. application Ser. No. 07/900,623, filed Jun. 18, 1992, now abandoned, which is a Continuation-in-part of U.S. application Ser. No. 07/628,111, filed Dec. 14, 1990, now U.S. Pat. No. 5,331,573.

FIELD OF THE INVENTION

The present invention relates to the design of compounds that mimic surface features of the endothelin family of peptides. In particular, cyclic peptides are provided that modulate that activity of endothelin. More particularly, cyclic peptides that specifically inhibit the activity of endothelin are provided.

BACKGROUND OF THE INVENTION

The vascular endothelium releases a variety of vasoactive substances, including the endothelium-derived vasoconstrictor prptide, endothelin (ET) (see, e.g., Vanhoutte et al. (1986) *Annual Rev. Physiol.* 48: 307–320; Furchgott and Zawadski (1980) *Nature* 288: 373–376). Endothelin, which was originally identified in the culture supernatant of porcine aortic endothelial cells (see, Yanagisawa et al. (1988) *Nature* 332: 411–415), is a potent twenty-one amino acid peptide vasoconstrictor. It is the most potent vasopressor known and is produced by numerous cell types, including the cells of the endothelium, trachea, kidney and brain. Endothelin is synthesized as a two hundred and three amino acid precursor preproendothelin that contains a signal sequence which is cleaved by an endogenous protease to produce a thirty-eight (human) or thirty-nine (porcine) amino acid peptide. This intermediate, referred to as big endothelin, is processed in vivo to the mature biologically active form by a putative endothelin-converting enzyme (ECE) that appears to be a metal-dependent neutral protease (see, e.g., Kashiwabara et al. (1989) *FEBS Lttrs.* 247: 337–340). Cleavage is required for induction of physiological responses (see, e.g., von Geldern et al. (1991) *Peptide Res.* 4: 32–35). In porcine aortic endothelial cells, the thirty-nine amino acid intermediate, big endothelin, is hydrolyzed at the Trp^{21}-Val^{22} bond to generate endothelin-1 and a C-terminal fragment. A similar cleavage occurs in human cells from a thirty-eight amino acid intermediate.

Three distinct endothelin isopeptides, endothelin-1, endothelin-2 and endothelin-3, that exhibit potent vasoconstrictor activity have been identified. The family of three isopeptides endothelin-1, endothelin-2 and endothelin-3 are encoded by a family of three genes (see, Inoue et al. (1989) *Proc. Natl. Acad. Sci. USA* 86: 2863–2867; see, also Thea et al. (1989)*J. Biol. Chem.* 264: 14613–14616). The nucleotide sequences of the three human genes are highly conserved within the region encoding the mature 21 amino acid peptides and the C-terminal portions of the peptides are identical. Endothelin-2 is (Trp^6, Leu^7) endothelin-1 and endothelin-3 is (Thr^2, Phe^4, Thr^5,Tyr^6, Lys^7, Tyr^{14}) endothelin-1. These peptides are, thus, highly conserved at the C-terminal ends.

Release of endothelin from cultured endothelial cells is modulated by a variety of chemical and physical stimuli and appears to be regulated at the level of transcription and/or translation. Expression of the gene encoding endothelin-1 is increased by chemical stimuli, including adrenaline, thrombin and Ca^{2+} ionophore. The production and release of endothelin from the endothelium is stimulated by angiotensin II, vasopressin, endotoxin, cyclosporine and other factors (see, Brooks et al. (1991) *Eur. J. Pharm.* 194:115–117), and is inhibited by nitric oxide. Endothelial cells appear to secrete short-lived endothelium-derived relaxing factors (EDRF), including nitric oxide or a related substance (Palmer et al. (1987) *Nature* 327: 524–526), when stimulated by vasoactive agents, such as acetylcholine and bradykinin. Endothelin-induced vasoconstriction is also attenuated by atrial natriuretic peptide (ANP).

The endothelins (referred to collectively as endothelin) or one or more endothelin peptides exhibit numerous biological activities in vitro and in vivo. Endothelin provokes a strong and sustained vasoconstriction in vivo in rats and in isolated vascular smooth muscle preparations; it also provokes the release of eicosanoids and endothelium-derived relaxing factor (EDRF) from perfused vascular beds. Intravenous administration of endothelin-1 and in vitro addition to vascular and other smooth muscle tissues produce long-lasting pressor effects and contraction, respectively (see, e.g., Bolger et al. (1991) *Can. J. Physiol. Pharmacol.* 69: 406–413). In isolated vascular strips, for example, endothelin-1 is a potent ($EC_{50}=4\times10^{-10}M$), slow acting, but persistent, contractile agent. In vivo, a single dose elevates blood pressure in about twenty to thirty minutes. Endothelin-induced vasoconstriction is not affected by antagonists to known neurotransmitters or hormonal factors, but is abolished by calcium channel antagonists. The effect of calcium channel antagonists, however, is most likely the result of inhibition of calcium influx, since calcium influx appears to be required for the long-lasting contractile response to endothelin.

Endothelin also mediates renin release, stimulates ANP release and induces a positive inotropic action in guinea pig atria. In the lung, endothelin-1 acts as a potent bronchoconstrictor (Maggi et al. (1989) *Eur. J. Pharmacol.* 160:179–182). Endothelin increases renal vascular resistance, decreases renal blood flow, and decreases glomerular filtrate rate. It is a potent mitogen for glomerular mesangial cells and invokes the phosphoinoside cascade in such cells (Simonson et al. (1990) *J. Clin. Invest.* 85: 790–797).

There are specific high affinity binding sites (dissociation constants in the range of $2–6\times10^{-10}M$) for the endothelins in the vascular system and in other tissues, including the intestine, heart, lungs, kidneys, spleen, adrenal glands and brain. Binding is not inhibited by catecholamines, vasoactive peptides, neurotoxins or calcium channel antagonists. Endothelin binds and interacts with receptor sites that are distinct from other autonomic receptors and voltage dependent calcium channels. Competitive binding studies indicate that there are multiple classes of receptors with different affinities for the endothelin isopeptides. The sarafotoxins, a group of peptide toxins from the venom of the snake *Atractaspis eingadensis* that cause severe coronary vasospasm in snake bite victims, have structural and functional homology to endothelin-1 and bind competitively to the same cardiac membrane receptors (Kloog et al. (1989) *Trends Pharmacol. Sci.* 10:21 2–214).

Two distinct endothelin receptors, designated ET_A and ET_B, have been identified and there is evidence that other subtypes exist (see, e.g., Emori et al. (1990) *FEBS Lett.* 263:261–264; and Sokolovsky et al. (1992) *J. Biol. Chem.* 267:20551–20554). DNA clones encoding the ET_A and ET_B receptors have been isolated (Arai et al. (1990) *Nature* 348: 730–732; Sakurai et al. (1990) *Nature* 348: 732–735). Based